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Citation: Review of Scientific Instruments **36**, 78 (1965); doi: 10.1063/1.1719332
View online: http://dx.doi.org/10.1063/1.1719332
View Table of Contents: http://scitation.aip.org/content/aip/journal/rsi/36/1?ver=pdfcov
Published by the AIP Publishing

Combined Calorimeter and Spectrophotometer for Observing Biological Reactions

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(Received 14 April 1964; and in final form, 17 September 1964)

A stopped-flow fast reaction apparatus is described which permits chemical reactions with rate constants of 50 sec^{-1}, or less, to be followed both spectrophotometrically and calorimetrically. The thermal detector is a single copper-constantan thermocouple, 5μ thick, with a response time of 3 msec. Using a bandpass of 0 to 80 cps, the signal-to-noise ratio is 1:1 for a heat change of 100 μcal/ml. The apparatus is housed in a 30×30-cm aluminum cylinder which is temperature stable to ±0.00001°C; reactants can be brought to within 0.001°C of equilibrium in less than 1 h. Acrylic light pipes are employed in the optical detection system to conduct a monochromatic light beam to the observation tube and to conduct the transmitted light to a photomultiplier. Solenoid valves control the mixer inputs; a third solenoid valve stops the flow in 2 msec. The thermal system and calorimeter were analyzed mathematically and two computer programs developed which make corrections for the response time of the system, correct the data for heat diffusion from the observation tube during the reaction, and calculate the first-order rate constant. The dehydration of carbonic acid was used as a test reaction. Experiments run at 3.8, 18, 24.1, and 36.9°C, using 0.04 molar $NaHCO_3$ and 0.02 *M* HCl gave results thermally of 3.12, 15.8, 22.7, 49 sec^{-1} and optically of 3.15, 15.1, 20.6, for the rate constant and 2100, 1413, 970, and 460 cal/mole for ΔH.

INTRODUCTION

THIS paper deals with the construction and analysis of a stopped-flow reaction apparatus for measuring rate constants and thermal changes in fast biological reactions. The flow method for measuring chemical reactions was first introduced by Hartridge and Roughton.[1] The method of operation involved the flowing of two reactants under pressure into a specially constructed mixing chamber from which the reacting solution then flowed up an observation tube where the reaction was observed spectrophotometrically. This basic procedure is still followed; however, advances have been made by Chance[2] from continuous flow to stopped and accelerated flow machines. Dirken and Mook[3] introduced a syringe-drive system, and the detection methods have expanded from the original photometric method to various schemes including thermoelectric, electrical conductivity, and electron spin resonance measurements. Roughton[4] has reviewed these advances in detail and gives references to the newer flow systems, detection methods, and applications. The instrument described here, which is an improved model of an earlier apparatus,[5] has a dual detection system which allows optical and thermal measurements to be made simultaneously. There are a number of advantages in this system; for example, in measuring the heats associated with the hemolysis of red blood cells,[6] simultaneous optical data is necessary in order to correlate the heats measured with the various stages of the reaction. In general the dual system affords more information about the reaction or reactions being observed, makes it easier to separate steps in a complex reaction, and allows a check of one method against the other. The major advantage of stopped flow over continuous flow in studying biological reactions is the economical use of difficult to obtain reactants, i.e., 100 ml of solution are required for 3 to 4 points in continuous flow, while only 5 ml are required for a complete record in stopped flow. Another aspect of the stopped-flow apparatus is the continuous monitoring of the reaction; this means that the mixing and stopping of the solution must be as rapid as possible, and that the detection systems must be fast compared with the reaction rate. The apparatus has an optical response of 5000 sec^{-1}. The speed is limited by the stopping time of the solution which is no faster than 2 msec. The thermal response time is 125 sec^{-1} due mainly to the galvanometer. The data obtained from reactions with rate constants larger than 10 sec^{-1} must be corrected to compensate for the response time of the thermal detection system. For reactions with rate constants of 0.5 sec^{-1} or less, thermal data must be corrected for heat loss from the calorimeter. The system is analyzed mathematically, and computer programs have been written which accept the raw data, automatically handle these two corrections, and compute the rate constant.

The temperature of the apparatus is thermostatically controlled in order that reaction rates may be measured over a wide temperature range. The system has been checked using the dehydration of carbonic acid, a well known reaction, as a system test. The results of these experiments are given.

[1] H. Hartridge and F. J. W. Roughton, Proc. Roy. Soc. (London) **A104**, 376 (1923).
[2] B. Chance, J. Franklin Inst. **229**, 455, 613, 737 (1940).
[3] M. N. J. Dirken and H. J. Mook, J. Physiol. (London) **70**, 373 (1930).
[4] F. J. W. Roughton and B. Chance, *Technique of Organic Chemistry*, edited by S. L. Friess, E. S. Lewis, and A. Weissberger (Interscience Publishers, Inc., New York, 1963), Vol. VIII, Part II, 2nd ed., Chap. XIV.
[5] R. L. Berger, *Temperature—Its Measurement and Control in Science and Industry* (Reinhold Publishing Corporation, New York, 1963), Vol. 3, Part 3, Chap. 6.
[6] L. C. Stoddart, Master's thesis, Utah State University (1962). Loss from monochromator to PMT is 80 dB in PMT output voltage; mainly at bends and observation tube surface of light pipe. Since this writing 1.6-mm Bausch & Lomb light wires 46 cm long have been added reducing the loss to 20 dB.

EXPERIMENTAL

A block diagram of the complete experimental setup is illustrated in Fig. 1. The internal parts of the apparatus are shown along with the 30×30-cm aluminum block thermostat in Fig. 2. Three 7-cm holes were bored through the cylinder; the outside holes contain the reservoirs, and the center hole contains the mixer-observation tube assembly. All the parts, including the observation tube, are designed so that they fit together with O-rings[7]; this greatly facilitates disassembly and replacement of parts.

The aluminum block with the apparatus mounted inside is placed in a water bath[8] which is regulated to ±0.01°C of the set temperature. The block with its large heat capacity then maintains thermal stability at the observation tube to better than ±0.00001°C. For accurate thermal measurements the solutions in the reservoirs should equilibrate to within ±0.001°C of each other before the experiment starts, and due to the rapid degradation of biological materials this equilibrium should be reached as rapidly as possible. By using the aluminum block with its large heat conductivity, this equilibrium can be reached in about 1 h.

The two reservoirs have inputs for compressed oxygen or other gas; this gas acts as the driving force for the reactants. Flow to the mixer is controlled by Valcor solenoid valves.[9] A third solenoid valve with a closing time of 2 msec was mounted on the output of the mixer and served to stop the flow. Alternatively, a stopping syringe, as shown in Fig. 3 may be used, this had the added advantage that the flow volume could be preset; and, since the flow time is accurately known from the recorded data, the flow velocity can be easily calculated for each experiment.

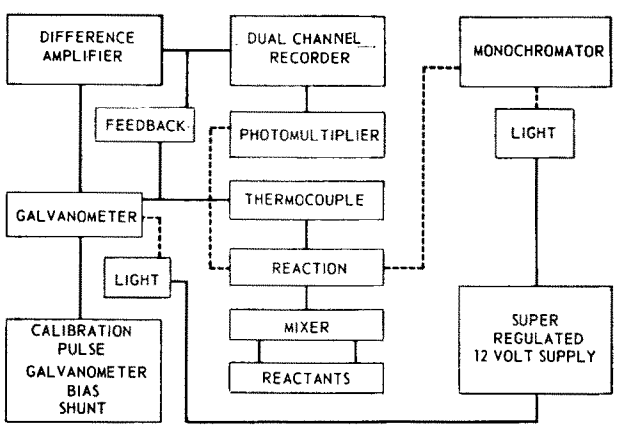

FIG. 1. Block diagram of the complete optical-thermal apparatus. A broken line indicates a light path.

[7] Parker Specialties, Inc., Alexandria, Virginia, Neoprene (N–219–7).

[8] American Instrument Company, Inc., Silver Spring, Maryland. Constant Temperature Bath No. 4–8605.

[9] Valcor Engineering Corporation, Kenilworth, New Jersey, type 491C194–Vitron diaphram.

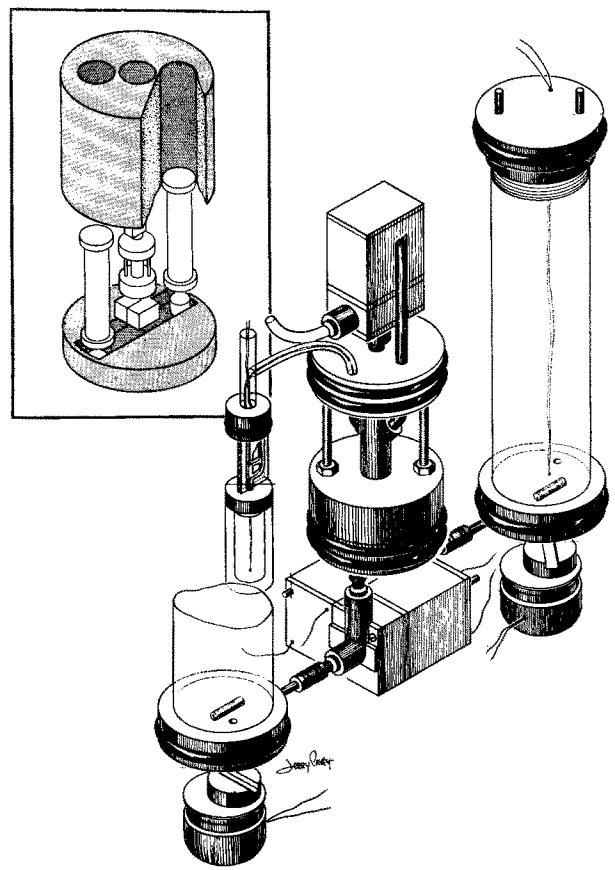

FIG. 2. An artist's drawing of the apparatus as it would look mounted in the block ready for operation.

Four- and ten-jet interchangeable mixers[10] were constructed for this apparatus. In order to obtain adequate mixing with high viscosity solutions, it is necessary to use the ten-jet mixer; however, a four-jet mixer was used for the experiments on the carbonate reaction discussed here, since all the solutions were at water viscosity. The inset of Fig. 3 shows the manner in which the mixer is made. A full description of the construction of multijet mixers will be published elsewhere.[10] The mixing efficiency was determined by mixing 2.0*M* HCl and 2.0*M* NaOH and measuring the temperature at various distances above the mixer with a thermocouple while the solutions were flowing. Since this is simply an ionization reaction, it is complete upon completion of mixing; therefore, the percentage of heat liberated at any time will also be the percentage of mixing at that time. Figure 4 is a plot of the results of this test. The curve shows that at the point of observation, which is 24 mm above the mixer, mixing is 98% complete, for the ten-jet mixer this point is reached in the mixing chamber. Four-jet mixers do not achieve mixing at these

[10] R. L. Berger in *Rapid Mixing and Sampling Techniques Applicable to the Study of Biochemical Reactions*, edited by B. Chance (Academic Press Inc., New York, to be published).

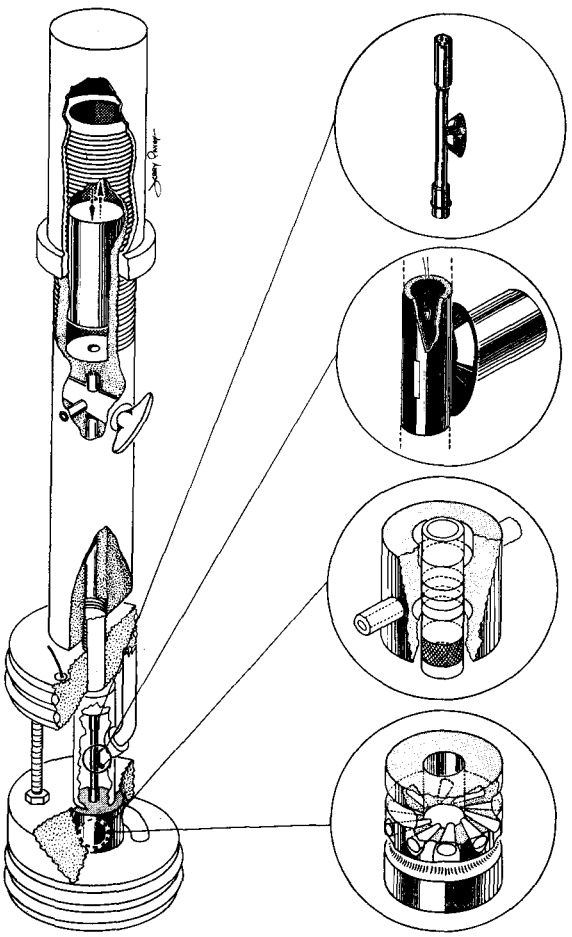

FIG. 3. Internal parts of the flow apparatus, with enlarged view of the mixer construction showing the jet arrangement, and enlarged view of the observation tube, showing the relative position of the thermal junction and the optical slit. A stopping syringe is shown which can be used in place of the solenoid valve.

flow velocities in the mixing chamber. Thus both Chance[4] and Gibson[11] have used multiple-jet or double mixers to attain adequate mixing in 1 msec.

The Pyrex observation tube (see Fig. 3 inset) had an i.d. of 3 mm and a wall thickness of 0.1 mm. Small O-rings were fitted to each end; these served to seal the observation tube in the output of the mixer and at the upper end of the tube where the thermocouple enters (see Fig. 2). The optical slits (2×1 mm) were formed on the observation tube itself by covering all but the slit area with a thin layer of flat black paint. The observation tube was enclosed in a hollow plastic cylinder 2.5 cm in diameter. This cylinder creates a dead air space around the observation tube which helps provide the proper temperature decay time (see below).

A single-junction thermocouple, inserted into the center of the observation tube, is used as the thermal detector. Unlike continuous flow apparatus, the response of the thermocouple in the stopped-flow thermal system is of para mount importance. It is necessary that the junction and insulation be as thin as possible. The thermojunction used was copper-constantan,[12] 5 μ thick with a very thin layer of G.E. 7031 Glyptal varnish as electrical insulation. The response of the thermojunction was measured by rapidly dipping the junction into ice water and monitoring the output on an oscilloscope. The response was found to be 1.2 msec to within $1/e$ of the total deflection (Fig. 5). The output of the thermocouple was connected to a special reflecting mirror galvanometer constructed by Downing.[13] With no feedback, this galvanometer has an 11-msec response time; in order to increase the response of the system, however, a feedback loop was used. This increased the response to about 7 msec. The circuit containing the galvanometer, feedback loop, bias, and calibration pulser is similar to that given by Hill[14] and later modified by Pinsent *et al.*[15] It is so constructed that a voltage in the form of a square pulse may be applied across the galvanometer at any time. The voltage was adjusted to approximate the thermocouple output from a temperature change of 0.01°C in the observation tube. This pulse was accurately calibrated by running experiments using known concentrations of HCl and NaOH producing known heats of reaction. The heats were recorded and compared with the

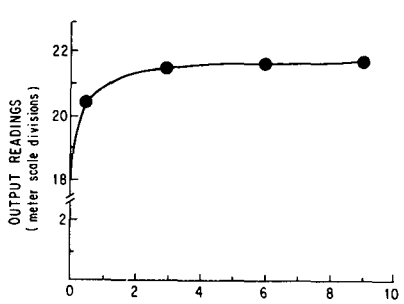

FIG. 4. Results of mixer efficiency test. The plot shows the relative heat measure from mixing HCl and NaOH as a function of distance up the observation tube. The plot shows that mixing is 98% complete at 2.4 cm up the tube.

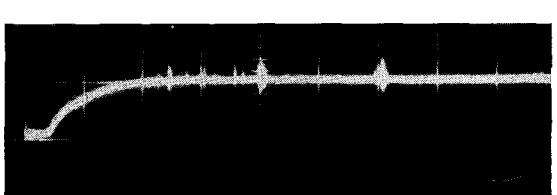

FIG. 5. Picture from an oscilloscope of the response of the thermojunction when it is rapidly dipped into ice water. The oscilloscope sweep is 2 msec/div, where the large divisions are 1 cm apart. The thermocouple reaches 90% of its output in 2 msec.

[11] Q. A. Gibson and L. Milnes, Biochem. J. **91**, 161 (1964).

[12] Science Products Corporation, Route 46, Dover, New Jersey.

[13] A. C. Downing, J. Sci. Instr. **25**, 230 (1948).

[14] A. V. Hill, J. Sci. Instr. **25**, 225 (1948).

[15] L. Pearson, B. R. W. Pinsent, and F. J. W. Roughton, Discussions Faraday Soc. **17**, 141 (1954).

height of the test pulse to determine its temperature equivalent. An alternate method for calibrating the pulse, which checks well with the above method, was to measure the test pulse voltage on a Keithley model 149 microvoltmeter. This is then compared with the volts per degree output of the thermocouple as measured on the microvoltmeter by changing the junction temperature from 25 to 0°C. By using this method, the test pulse was found to be equivalent to 0.0146°C. Once the pulse was calibrated, it could be applied during an experiment to obtain the thermal sensitivity of the apparatus. Also, by using the bias circuit, the galvanometer zero point can be moved across the recording paper and the test pulse applied at various points to check the linearity of the thermal system. A twin photoresistive Clairex cell[16] and a difference amplifier were employed to detect the motion of the light beam from the galvanometer. Details are given in Ref. 5. The output goes to one channel of a dual channel Offner RS dynograph recorder.

The light source for the optical system was a G.E. 1074 12-V bulb in a lens system which columnated and focused the light on the entrance slit of a Farrand UVIS monochromator. The output of the monochromator was focused on the end of a light pipe made of 6-min acrylic rod, which conducted the monochromatic light beam to the observation tube (see Fig. 2). The light transmitted through the observation tube was conducted by a second light pipe to a 1P21 photomultiplier, the output of which goes to the second channel of the recorder. The photomultiplier was operated at 80 V per dynode using a 220-kΩ load resistor shunted by 0.002 μF for filtering high frequency noise. The dc balance of the RS Offner recorder was used as a bucking voltage.

The power supply for the galvanometer light beam and the monochromator light source was a regulated 12-V commercial supply with a super-regulator specially constructed to have very low noise and a fast transient response. Ripple was less than 5 μV peak-to-peak and the jitter was less than 10 μsec. Random transients above 500 cycles were less than 20 μV peak-to-peak. The transient response for recovery from no load to full load was 120 μsec. The drift was 1 ppm/min. Since a complete set of runs is made in less than 5 min, this was adequate. Chopper stabilization of the supply could be used with some rise in noise level but considerable reduction in drift.[17]

Since the thermocouple output will generally be in the range of 10^{-6} to 10^{-8} V, it was necessary to build a special galvanometer mount to reduce the noise produced by motion of the galvanometer mirror which resulted from building vibration. The most efficient mounting we found, as illustrated in Fig. 6 was composed of a double inner tube suspension[18] inside an acoustically insulated box mounted on cement blocks and lead sheets. The main interference was 14 and 28 cycles, with possible other harmonics, coming from the building air conditioning system.

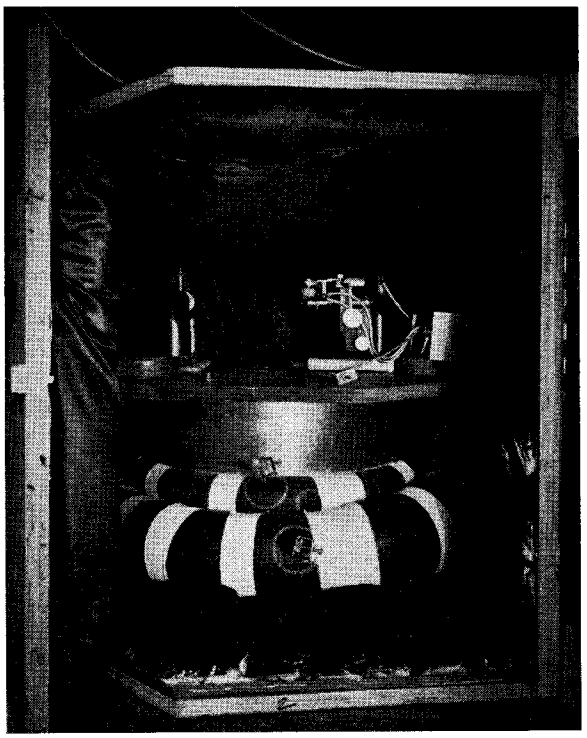

FIG. 6. Galvanometer, light source, and detector on the galvanometer mount which is composed of 136 kg of lead shot on a double inner tube suspension. The mount is inside an acoustically insulated box mounted on cement blocks and lead sheets.

METHODS

Analytical reagents were utilized for all the work discussed in this paper. All solutions for the carbonate reaction studies, including the optical calibrating solutions, were made up with 0.005% bromophenol blue, added as the *p*H indicator. The HCl and NaOH standards and the bromophenol blue were obtained from British Drug House. Optical measurements were made at 590 mμ.

The carbonate reaction was observed utilizing three different concentrations of HCl and $NaHCO_3$; each of the three experiments was repeated three times at four different temperatures. The concentrations before mixing were 0.08, 0.04, and 0.01*M* HCl and 0.16, 0.08, 0.02*M* $NaHCO_3$, respectively; the temperatures were 3.8, 18, 24.1, and 36.8°C. In performing an experiment, the reactants are brought to ±0.1°C of each other and then poured into their respective reservoirs and allowed to come to equilibrium. A thermocouple is employed to monitor the temperature difference between the reservoirs; when this dif-

[16] Clairex Corporation, New York, type Cl-7.

[17] R. L. Berger, J. M. Peterson, and E. Budge, Rev. Sci. Instr. **36**, 93 (1965).

[18] A. Elliott and J. Home, *Laboratory Instruments; Their Design and Application* (Chemical Publishing Company, Inc., New York, 1953), pp. 163–64.

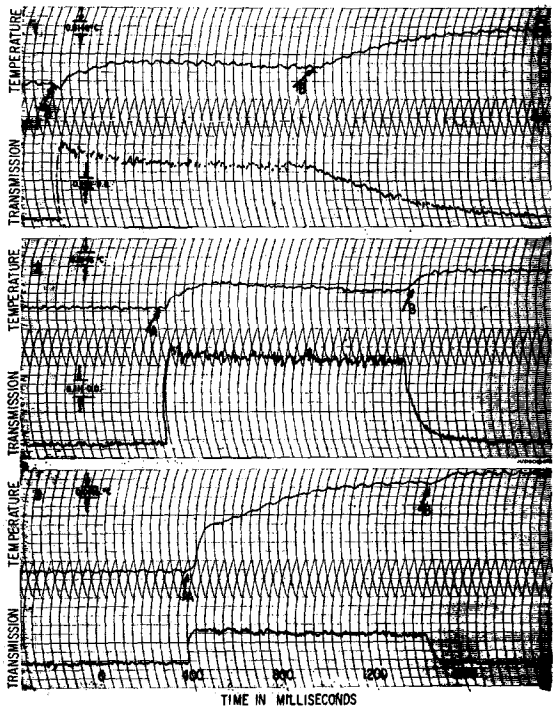

FIG. 7. Three examples of raw data from the carbonate reaction experiments. The top curve in each of the three recordings is the thermal response, the lower curve, the optical response. The concentrations of reactant are 0.08 M in $NaHCO_3$ and 0.04 M in HCl for (1) and (2), and 0.16 M in $NaHCO_3$ and 0.08 M in HCl for (3), only the temperature is varied in the three examples; (1) is at 3.8°C, (2) at 24°C, and (3) at 36.9°C. Chart speed is 40 msec/div; time increasing from left to right. The arrows indicate the time when flow started and stopped.

ference is approximately 0.001°C, (i.e., about 1 h), the run is started. The solutions are allowed to flow for approximately 1 sec, at which time the flow is abruptly stopped and the optical and thermal changes observed. After the heat produced by the reaction decays from the system, the flow is repeated. This procedure can be repeated up to 20 times. Examples of the raw data taken at three different temperatures are shown in Fig. 7. The noise on the optical curve is mainly due to the photomultiplier tube operating at a fairly low light level due to the high loss through the light pipe system. This has since been replaced by Bausch and Lomb 1.6-mm light wires with a gain of a thousand so that no noise exists at these sensitivities. The thermal noise arises mainly from the building vibration and a small electro kinetic effect at the thermocouple tip. The insulation must be as thin as possible for fast response, thus some noise must be tolerated. Recently a very quiet room has been found and the noise level is down a factor of ten from that presented here.

In performing an experiment samples of the reacted mixture of HCl and $NaHCO_3$ are collected and the mixing ratios determined by titation. The ratios were $1:1\pm6\%$ for all the experiments included. The flow velocity was calculated by dividing the volume flow per second by the cross-

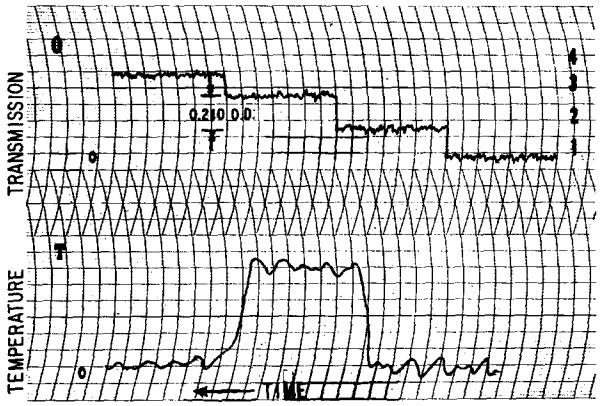

FIG. 8. A representative example of an electrically simulated thermal calibration pulse (bottom) and an optical calibration (top). The time scale on the thermal pulse is 40 msec/div and 1 sec/div on the optical. The pulse represents a Δt of 0.0146°C. The pH values of the four optical calibration solutions 1, 2, 3, and 4, are 3.530, 3.690, 3.900, and 4.020; wavelength is 590 mμ with a 3-mm light path.

sectional area of the observation tube. The volume flow per second was ascertained by flowing for a known period of time as determined with a stop watch, and measuring the volume of the collected efflux. The flow velocity used was 400 ± 20 cm/sec which is well above the Reynolds' number for turbulent flow.

The pH sensitivity of the optical system was calibrated after each run, using four standard solutions ranging from pH 3.5 to 4. The standards, prepared from 0.1M acetic acid and sodium acetate solutions, were flowed one at a time into the observation tube and an optical reading was taken at 590 mμ. Samples were collected from the apparatus and the pH measured on a Beckman expanded scale Zeromatic pH meter. An example of the recorded thermal test pulse and optical calibration is shown in Fig. 8.

DISCUSSION AND RESULTS

In the carbonate experiments, the following reaction scheme has been proposed[19–22] when HCl and $NaHCO_3$ are mixed:

$$\begin{array}{ccc} H^+ + HCO_3^- & \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} & H_2CO_3 \\ k_{13}\Big\| k_{31} & & k_{32}\Big\| k_{23} \\ CO_2 + H_2O & & \end{array} \qquad (1)$$

The first reaction following the mixture of H^+ and HCO_3^- had a rate constant of 10^8 sec^{-1} [19] which is immeasurably fast for this system and is seen as a very rapid rise in the thermal measurement. No optical change occurs. In

[19] M. Eigen, K. Kustin, and G. Mass, Z. Physik. Chem. **30**, 130 (1961).
[20] J. T. Edsall and J. Wyman, *Biophysical Chemistry* (Academic Press Inc., New York, 1958), Vol. 1, Chap. 10.
[21] B. H. Gibbons and J. T. Edsall, J. Biol. Chem. **238**, 3502 (1963).
[22] C. Ho and J. M. Sturtevant, J. Biol. Chem. **238**, 3499 (1963).

the pH range in which we are working, i.e., between pH 3.5 and 5.5, a second reaction occurs which has been taken to be

$$H_2CO_3 \overset{k}{\rightleftharpoons} CO_2+H_2O. \quad (2)$$

There is, however, some doubt as to the mechanism of (1) so that one can only say that a second reaction occurs which can be observed optically by following the H^+ concentration, and thermally as a result of the accompanying temperature change. Brinkman[23] has shown that under these pH conditions the rate constant k for (2) is given, in terms of the hydrogen ion concentration, as

$$(t_2-t_1)k=2.3[1+K_{H_2CO_3}/(b-a)]\log H_2^+/H_1^+,$$

where b is the concentration of $NaHCO_3$ and a is the concentration of HCl.

In terms of pH, this becomes

$$k=2.3[1+K_{H_2CO_3}/(b-a)][(pH_2-pH_1)/(t_2-t_1)], \quad (3)$$

where $K_{H_2CO_3}$ is the true first dissociation constant. This equation was used to determine k from the optical data. The thermal system was following the breakdown of H_2CO_3 to CO_2+H_2O which is, to the first approximation, first order; therefore

$$C=C_0e^{-kt},$$

where C in this case is the concentration of H_2CO_3. If this equation is normalized, and C is expressed in terms of the heat of reaction Y, i.e., $C=1-Y$,

$$Y=1-e^{-kt}. \quad (4)$$

As was stated above, the data must be corrected to compensate for the response of the detection systems. The error is negligible only when the response of the detection system is fast compared to the rate of the reaction occurring. Sirs[24] has shown, for example, that significant error is encountered even when the response of the detection system is a factor of 10 or more faster than the reaction rate constant. We, therefore, set up a simple computer program to make these corrections and calculate the rate constant.

An equation for the response of the thermal system to the first-order rate equation $1-e^{-kt}$ was derived using the method of Laplace transforms. To find the combined response of components in series, i.e., the thermocouple, galvanometer, amplifier, and recorder, the network transforms[24] of each component are multiplied together. This is then multiplied by the transform of the first-order rate equation, and the inverse taken. This inverse becomes quite involved, but finally reduces to

$$E_{out}=1+K_1e^{-at}+K_2e^{-bt}+K_3e^{-ct}+K_4e^{-dt}+K_5e^{-kt}+K_6te^{-at}, \quad (5)$$

where the K's are rather complicated functions of a, b, c, d, and k; however, except for k, these are all known response time constants of the system. Details are given in Ref. 25.

Equation (5) was programmed for the IBM 1620 computer. [See Ref. 25.] In the program a, b, c, d, and k were left as independent variables. When values for these variables are entered, the computer calculates the value of E_{out} at various times. Thus a continuous plot of Eq. (5) can be made and compared with a plot of the input equation, $1-e^{-kt}$. This program was developed primarily to show distortion in the output signal as compared with the input. In practice Eq. (5) is solved for k, the unknown rate constant. This is possible since everything else in the equation is known including E_{out} for all values of time. E_{out} in this case is the recorded data.

A slight complication exists in solving for k. Since all the coefficients of the exponentials in Eq. (5) are functions of k, it makes it impossible to solve for k directly.

To eliminate this difficulty, the program is designed so that it computes E_{out} at some time t_1 as before, using an initial estimate for k. E_{out} computed is then compared by the computer with the value of E_{out} at t_1 taken from the experimental data.

The optical data also required correction but this was relatively simple, as it was necessary only to correct for the response of the recorder; the photomultiplier circuit was rapid enough that it offered no significant error. Since the optical output on the recorder was calibrated in pH units, Eq. (3) can be written $E_{out}=-kt/2.44$, where $E_{out}=pH_2-pH_1$, $t_1=0$, and $K_{H_2CO_3}/(b-a)=0.06$. The Laplace transform of this, $k/2.44s^2$, multiplied by the recorder network transform $a/(s+a)$ gives $ka/2.44s^2(s+a)$. The inverse is $(k/2.44)[t-(1-e^{-at})/a]=E_{out}$, or

$$k=2.44E_{out}[a/at-(1-e^{-at})]. \quad (6)$$

Equation (6) was programmed for the computer; the values of E_{out} and t from the data were used as input data to obtain the corrected value of k. The analysis indicated that such corrections are necessary with rate constants faster than 5 sec^{-1}.

Thermal data from reactions with rate constants of 1 sec^{-1} or less must be corrected for heat conduction from the observation tube. A program was developed which makes a transient analysis of the heat conduction losses with distributive sources in the observation tube. A subroutine takes the measured temperature, corrects them for

[23] R. Brinkman, R. Margaria, and F. J. W. Roughton, Trans. Roy. Soc. (London) A232, 65 (1933).
[24] J. S. Sirs, Ph.D. thesis, Cambridge University (1955).
[25] L. C. Stoddart and R. L. Berger, Rev. Sci. Instr. 36, 85 (1965).

TABLE I. Carbonate dehydration rate constant.

Temp. (°C+0.1)	Conc. $NaHCO_3$[a] (moles/liter)	ksec^{-1} Optical[b]	Std	Thermal	Std	No. of experiments Optical	Thermal	H (cal/mole)
3.8	0.08	2.49	0.31	3.13	0.29	7	8	2100
	0.04	3.15	0.32	3.12	0.26	6	8	
	0.02	2.48	0.11	2.79	0.26	4	4	
18.0	0.04	15.11		15.80	1.5	2	3	1413
24.1	0.08	25.89	1.88	21.77	0.59	7	8	970
	0.04	20.60	1.21	22.70	2.96	6	8	
	0.01	19.00	1.98	17.1	3.15	6	6	
36.9	0.04			49.04	9.08		6	460

[a] HCl concentration, after mixing, one half that of $NaHCO_3$.
[b] 590 mμ.

conduction losses and then calculates the rate constant. Details are given in another paper.[26]

For the carbonate reaction used to test the system, rate constants were found to be within the range of 2.4 to 50 sec^{-1}; therefore, the first computer program was used. Table I tabulates the data taken with the optical-thermal apparatus. Table II shows thermal data recorded by Roughton[27] and the optical data of Dalziel.[28]

The purpose of studying this reaction was to demonstrate the abilities and limitations of this apparatus, particularly the use of thermocouple detection of fast reactions in a stopped-flow machine. From Table I it can be seen that the thermal system is limited to reactions slower than 50 sec^{-1} due to the galvanometer response. Work is progressing on a system to eliminate the galvanometer and also increase the speed of response of the thermocouple. In order to utilize the faster response of the optical system one must move nearer the mixing chamber, use a smaller observation tube and increase the flow rate. All of these can, of course, be quite simply done when the need arises.

TABLE II. Carbonate dehydration rate constant from the literature.

Temp. (°C)	Thermal[a] (ksec^{-1})	ΔH (cal/mole)	Optical[b] (ksec^{-1})
0.0	1.79±0.09	2775	
17.8	14.6 ±0.8	1420	12.3±0.4
27.0	31.0 ±2	1040	
36.7	80.0 ±7	450	

[a] Roughton.
[b] Dalziel 18°C.

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ACKNOWLEDGMENT

It is a pleasure to acknowledge the assistance and encouragement in this work received from Professor F. J. W. Roughton, F.R.S., Department of Colloid Science, Cambridge University. His guidance in the reaction kinetics is particularly appreciated. Financial assistance in the early phases of this work while the authors were in the Department of Physics, Utah State University, Logan, Utah was received from the University Research Fund, Grant U-46, and Grant A-2601 from the National Institutes of Health.